Study Protocol and Statistical Analysis Plan

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Clinical and Microbiological Evaluation of the Chemo-mechanical Caries Removal Agent (BRIX3000®) in Primary Molars: A Randomized Controlled Clinical Trials.

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Clinical and Microbiological Evaluation of the Chemo-mechanical Caries Removal Agent (BRIX3000®) in Primary Molars: A Randomized Controlled Clinical Trial.

التقييم السريري والميكروبيولوجي للعامل الكيميائي- الميكانيكي لإزالة تسوس الأسنان (BRIX3000®) في الأضراس الأولية: تجربة سريرية محكمة عشوائية.

By

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BDS, 2004; MSc,2012

Thesis Proposal Submitted in Partial Fulfillment of the Requirement for the Degree of Doctor of Philosophy in Pediatric Dentistry (PhD)

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RESEARCH PROBLEM

Dental caries is one of the most prevalent chronic diseases of people worldwide. It results in localized dissolution and destruction of the calcified tooth tissues (Selwitz et al., 2007). Dental caries has been considered as a great challenge for the dentist, as they seek for efficient means to control it. The destruction to the dental structure does not develop as a result of localized accumulation of bacteria, rather it is an invasive and infectious process, which result from the interaction of multiple inter-related factors (Lima et al., 2005). The "caries-affected" dentine is described as demineralized inter-tubular dentin, crystal deposition in dentinal tubules, lesser amount of destructed collagen matrix with no bacterial invasion. On the contrary, the "caries-infected" dentine displays deformation of the microstructure of the dentinal tubules, permanently-damaged collagen fibers with significant penetration of the bacteria. Thus, the "caries-infected" dentine has to be removed only during caries removal (Hamama et al., 2014).

Traditional clinical treatment of dental decay has developed on the basis of removal of carious tissues with high-speed handpieces and slow-rotating instruments inducing pain, disturbing sounds and vibrations. In addition, this method comprises the tooth structure by removing both "caries-affected dentin" and "caries-infected tissues" (Jawa et al., 2010).The concept of minimal intervention dentistry includes the selective removal of caries-infected tissue with preserving the healthy caries-affected tissue that have the ability of remineralization. The use of chemo-mechanical caries removal is one of the main implementations of the minimum intervention dentistry idea accepted in the last ten years. "Chemo-mechanical caries removal" requires the chemical mellowing of decayed dentin then its elimination by light removal using hand instruments (Hamama et al., 2014). Chemo-mechanical caries removal differs from the conventional surgical by that it selectively removes carious dentine thereby it is less destructive and less painful promoting a positive attitude of visiting dentists among the children (Goomer et

al., 2013). Investigations are required to evaluate and compare the outcome of two chemo-mechanical agents (BRIX3000®) and (Carie-Care™) versus conventional surgical methods. Therefore, the present study aims to perform clinical and microbiological evaluation of caries removal using (BRIX3000®) and (Carie-Care™) versus conventional surgical methods in primary molars among children in Jeddah city, Saudi Arabia.

LITERATURE REVIEW

Rotary systems including high- and low-speeds air rotors are usually used for caries removal. Theses rotary devices has the advantage of rapid caries removal. However, it has the disadvantage of jeopardizing the healthy tooth structure, uncomfortable, and the possibility of pulpal injury as a result of heat and pressure created during preparation (Maragakis et al., 2001).

Novel techniques have been developed as alternatives to conventional methods for caries removal in pediatric dentistry. The chemo-mechanical caries removal technique though it is more comfortable it requires more time with anticipation to conventional surgical methods for caries access and finishing the preparation (Soni et al., 2015). Several "chemo-mechanical caries removal agents" have been developed in the market since 1975. They can be generally categorized into either sodium hypochlorite (GK-101, GK-101E, Caridex®, Cariosolv®) (Fig.1) or enzyme-based agents (Papacárie®, Carie-Care™, Brix3000®) (Fig.2) (Hamama et al., 2014, Venkataraghavan et al., 2013, Felizardo et al., 2018).

Many investigations have compared the efficacy, time taken, and pain threshold of different chemo-mechanical agents versus the conventional surgical method.

Regarding the efficacy of caries removal, Goomer et al. (2013) concluded that chemo-mechanical method for removal of decay with Cariosolv was less effective than traditional surgical technique though it was a worthwhile alternative to the painful procedures; like drilling, especially among children, who are afraid of dentists. These results were confirmed by other studies conducted by "Pandit et al. (2007), Bohari et al. (2012) and Soni et al. (2015)". Other studies conducted by "Fure et al. (2000), Banerjee et al. (2000), Bergmann et al. (2005), Lozano-Chourio et al. (2006), Peric et al. (2009) and Almaz et al. (2016), Kochhar et al. (2011)" showed that surgical and chemo-mechanical method exhibits similar efficacy to remove caries.

Maragakis et al. (2001) and Yazici et al. (2005) concluded that Cariosolv could not be an alternative to surgical method since the caries was not removed completely.

Regarding the time taken of caries removal, several studies found that chemo-mechanical methods needed significantly greater time compared to surgical caries removal. This is most likely because chemo-mechanical agents usually require frequent implementation for caries removal (Goomer et al., 2013, Soni et al., 2015, Banerjee et al., 2000, Pandit et al., 2007, Kochhar et al., 2011, Maragakis et al., 2001, Fure et al., 2000, Bergmann et al., 2005, Lozano-Chourio et al., 2006, Peric et al., 2009).

Regarding the pain experienced during caries removal, several studies concluded that chemo-mechanical caries removal is more accepted, less painful technique of caries removal allowing patient to participate positively during dental treatment compared to the surgical method (Soni et al., 2015, Goomer et al., 2013, Pandit et al., 2007, Kochhar et al., 2011, Rafique et al., 2003). This finding could be attributed to many factors. Anusavice and Kincheloe (1987) explained absence of pain or mild pain sensation associated with chemo-mechanical caries removal because only carious dentin is removed in contrast to the conventional surgical method that includes removal of both sound and carious dentin. In addition, chemo-mechanical agents are available in form of gel covering the cavity that can provide thermal insulation during the caries removal. Meanwhile, no vibration or heat generation during the use of chemo-mechanical method resulting in no or little opening of dentinal tubules that can be account for minimal pulpal response. Munshi et al. (2002) mentioned that chemo-mechanical caries removal does not persuade pain and anxiety because it does not call for the use of local anesthesia thus avoiding the trauma associated with the injection and the fear of the needle.

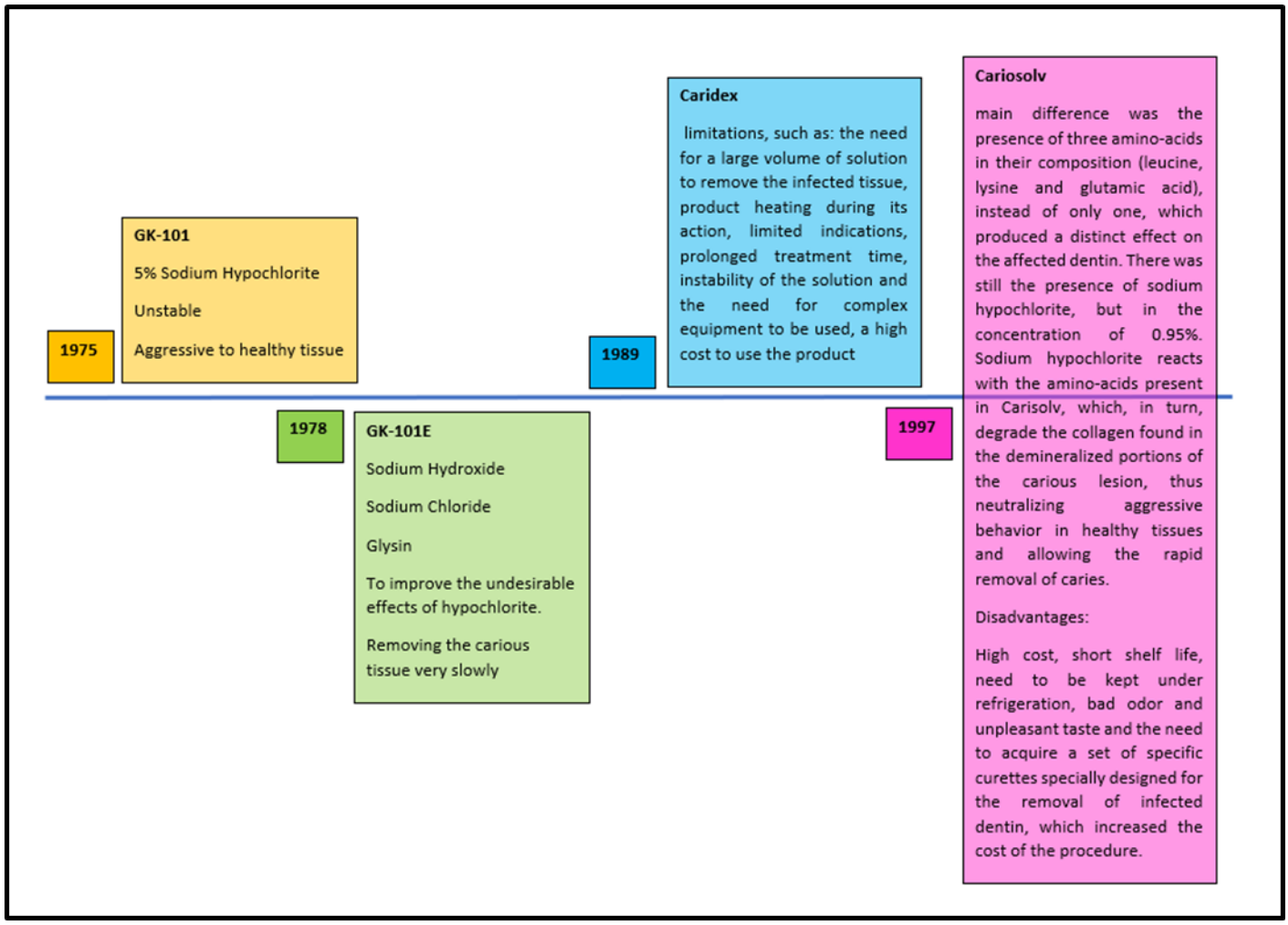

Figure 1: Sodium hypochlorite-based chemo-mechanical caries removal agents.

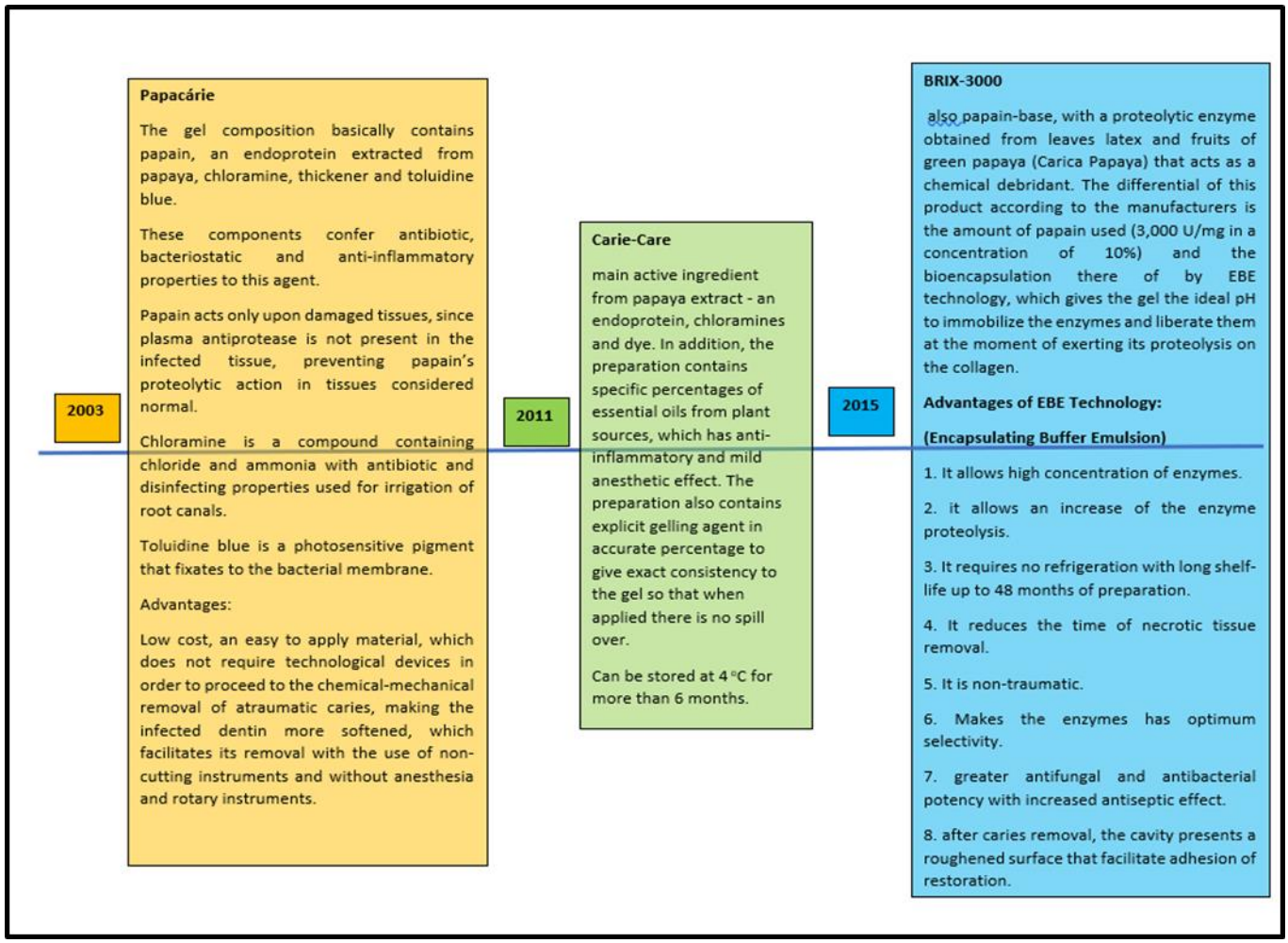

Figure 2: Enzyme-based chemo-mechanical caries removal agents.

IMPORTANCE OF THE RESEARCH

The disadvantages accompanied with the use of the conventional surgical method of caries removal has been overcome by the use of "chemo-mechanical caries removal method". "Chemo-mechanical caries removal" is one of the minimally invasive techniques that found to be less painful, less destructive and more accepted by children (Goomer et al., 2013, Soni et al., 2015). In addition to these advantages, the research holds clinical significance as a novel agent for "chemo-mechanical caries removal" (BRIX3000®) by the laboratory Brix Medical Science. It has been released recently in the dental market in 2015. It is a papain-based gel derived from leaves latex and fruits of green papaya (Carcia Papaya) that works as chemical debridement. It is for atraumatic caries removal with unique technology Encapsulating Buffer Emulsion (EPE) (Felizardo et al., 2018). This technology, according to the manufacture, provides the product with:

1. Proteolytic enzymatic activity that differs than previous products (Carie-Care) in the amount of papain used (3,000 U/mg in a concentration of 10%).
2. Perfect pH that solidifies the enzymes and set them free instantly on the collagen through its proteolytic activity.
3. Stable in room temperature with no need to be refrigerated with increased its shelf-life up to 48 months.
4. Increased enzymatic selectivity to damaged collagen.
5. Reduced time needed for removing the damaged dentinal tissues.
6. Enhanced the antifungal, antibacterial, and antiseptic ability.
7. Increased roughness to the cavity floor that favored better adhesion of the restoration.

By reviewing the literature, there are limited studies concerning its implementation in the area of pediatric dentistry. Clinical and microbiological studies are needed to confirm

manufacturer's claims by comparing this novel chemo-mechanical agent (BRIX3000®) and the previously-available chemo-mechanical agent (Carie-Care™) to the conventional surgical method for caries removal. Therefore, the purpose of this research is to perform clinical and microbiological evaluation of caries removal using these three different methods of caries removal in primary molars.

RESEARCH OBJECTIVES

1) To compare the efficacy of the chemo-mechanical method (BRIX3000®, Carie-Care™) versus the conventional surgical method in decreasing the cariogenic bacteria in dentine caries of primary molar teeth in vivo.

2) To compare the efficiency of the chemo-mechanical method (BRIX3000®, Carie-Care™) versus the conventional surgical method in caries removal of primary molar teeth in vivo.

3) To compare the pain experienced by children during dental treatment with the chemo-mechanical method (BRIX3000®, Carie-Care™) versus the conventional surgical method in caries removal of primary molar teeth in vivo.

HYPOTHESIS

The null hypothesis for this research will be as follows:

There is no difference in the efficacy, efficiency and pain experienced between chemo-mechanical caries removal agents (Brix3000®), (Carie-Care™) and the conventional surgical methods in primary molars.

RESEARCH MATERIALS AND METHODS

This study will be will be carried out at King Abdulaziz Dental University Hospital (KADUH) and at Microbiology Research Laboratory in the Faculty of Pharmacy, King Abdulaziz University in Jeddah, Saudi Arabia to evaluate the efficacy, efficiency, pain experienced by children with the use of the chemo-mechanical method (BRIX3000®) and (Carie-Care™) versus the conventional surgical method in primary molars.

Materials:

Ethical Approval

The study was approved by the "Research Ethics Committee" at Faculty of Dentistry, King Abdulaziz University (KAU), under approval number 107-06-19.

Informed Consent

Written informed consent will be received from the parents or the guardians of the children after explaining the research procedures to them.

Subjects (sampling)

The study sample will consist of healthy and cooperative children between the ages of 4-9 years with bilateral open occlusal carious lesions in primary molars, who will attend as outpatients to the Department of Pediatric Dentistry at KADUH during the period from October 2019 to October 2020.

Inclusion criteria:

- Healthy and cooperative patients of age group 4-9 years.
- Primary first/second molars with open occlusal carious lesion having moderated depth and clinically visible brownish color softened dentin.
- No clinical signs or symptoms of pulp degeneration, such as tenderness to percussion and history of sinus tracts or swelling. Intraoral periapical radiographs with lesions having radiolucency extending into, but only confined to dentin.

Exclusion Criteria:

- Patients with any major and minor systemic illness.
- Uncooperative patients that necessitate pharmacological dental treatment.
- Primary molars with mobile teeth, arrested caries, restored teeth, non-vital teeth, no carious lesion, presence of developmental defects and non-restorable teeth.
- Patients on any antibiotic regimen either on the day of treatment or for at least 2 weeks prior to the study.
- Patients allergic to Latex.
- Radiographic evidence of external or internal root resorption, furcal or periapical radiolucency.
- Primary molars with more than half of the root length resorbed.
- Children who did not attend and complete the second session of treatment.
- Not approving to sign the consent.

Sample Size Calculation:

The sample size was measured using G power analysis for calculating estimated sample size. Based on the results of Bohari et al. 2012, it was found that 30 subjects per group were required

to detect a statistically significant difference between the two groups anxiety score (FLACC) with a power of 95% at the significance level of 0.05.

The study group will be consisted of 120 primary molars with bilateral design from 60 children aged 4-9 years without sex predilection. The included teeth will be assigned randomly into one of the following groups according to the decay removal technique that will be utilized using two-arm parallel groups with split-mouth design:

The control group (A): (n=60) primary molars will be subjected to removal of caries using a conventional drilling method using carbide dental burs.

The test group (B): (n=30) primary molars will be subjected to removal of caries using a chemo-mechanical agent (Brix3000®, Brix Medical Science, Carcarañá-Santa Fe, Argentina).

The test group (C): (n=30) primary molars will be subjected to removal of caries using a chemo-mechanical agent Carie-Care™, Uni-Biotech Pharmaceuticals Private Limited, Chennai, India in collaboration with Vittal Mallya Scientific Research Foundation).

Armamentarium & Equipment

1) Basic dental examination Kit
2) Restorative kit
3) Brix3000® "Brix Medical Science, Carcarañá-Santa Fe, Argentina"
4) Carie-Care™ "Uni-Biotech Pharmaceuticals Private Limited, Chennai, India in collaboration with Vittal Mallya Scientific Research Foundation".
5) Dental bur "Low-Speed Tungsten Carbide round bur US-NO 1-8, Meisinger, USA. L.L.C., Easter Avenue Centennial, Colorado; High-Speed Diamond round medium size 08-018, Meisinger, USA. L.L.C., Easter Avenue Centennial, Colorado"

6) Rubber dam kit.

7) Sterile vial.

8) Bacteriological incubator

9) Inoculation loop.

10) Blood agar plates “Schaedler agar”

11) GC Fuji IX GP®.

12) A Stop Watch.

Methods:

Study design

Experimental, randomized controlled clinical trial with two-arm parallel groups and bilateral design following the “Consolidated Standards of Reporting Trials (CONSORT,2010)” statement (Fig.3).

Randomization

Since bilateral technique will be utilized in both parallel groups, it was decided to start on the right side irrespective of the method assigned by randomization. To control the variable of the side favored by the clinician, the researchers will make sure that each side is treated evenly by both methods using block randomization. Before recruiting participants, 60 sealed envelopes containing the randomization results will be arranged, sealed and mixed blindly in a box. Each envelope represents a block of the two contralateral teeth. The envelopes will be numbered blindly from 1 to 60. Each envelope will be opened immediately before starting the procedure on the right side.

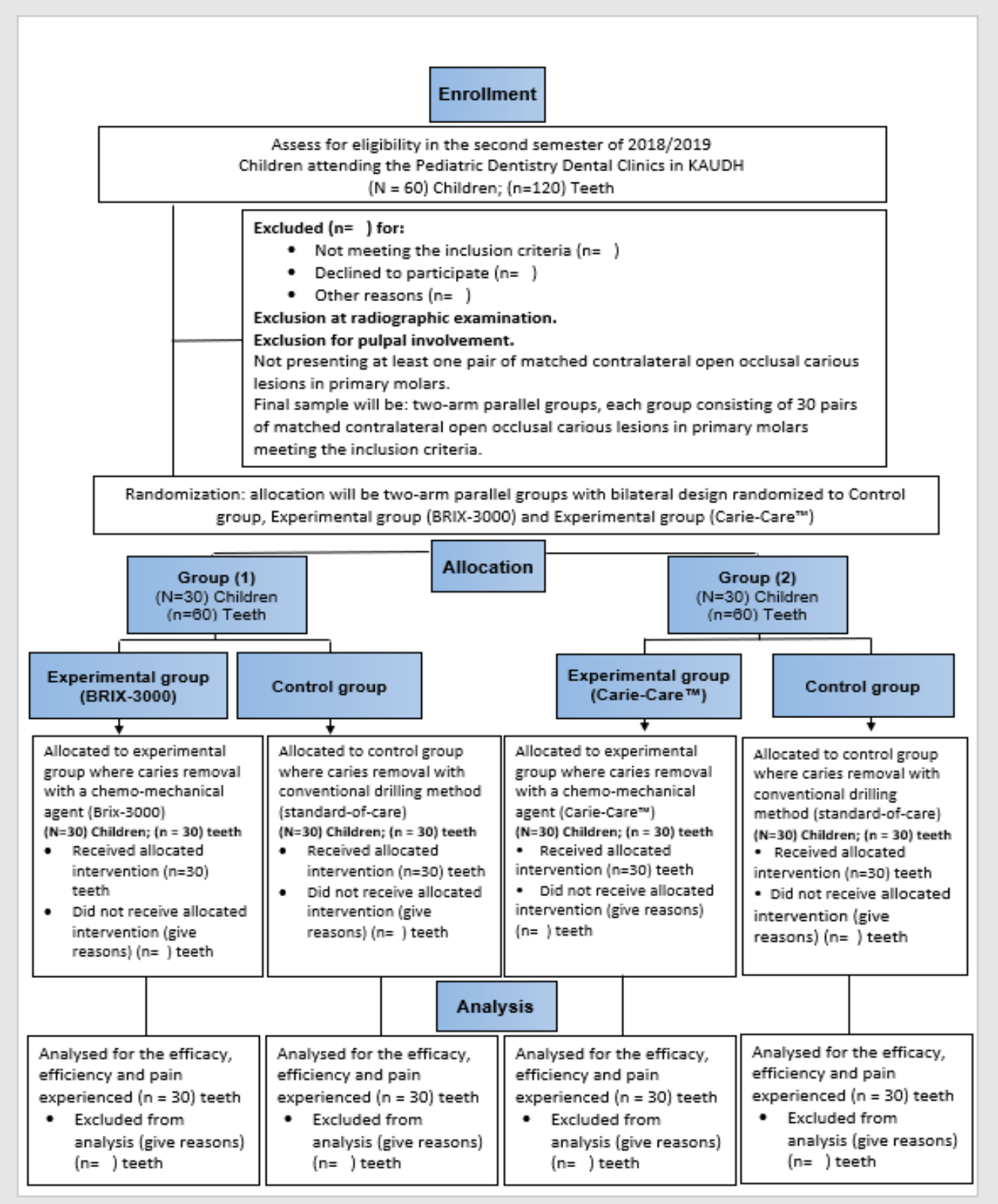

Figure 3: Flow diagram of the Study

Clinical Procedures

The degree of dental destruction will be clinically determined by using a dental probe. Only open occlusal cavities with brown and softened dentin with broadly comparable depth exhibiting frank moderate cavitation will be chosen "severe decay, stage 5, ICDAS [International Caries Detection and Assessment System]" (Ismail et al., 2007, Dikmen, 2015). The depth of caries progression will be confirmed with an intraoral periapical radiograph such that radiolucency did not involve the pulp.

To assess the efficacy of caries removal in all groups, the outer surface of the decayed lesion will be washed with sterile normal saline (5 mL) to avoid contamination of plaque bacteria before taking the sample. The first sample will be taken from superficial carious lesion with the aid of a sterile sharp spoon excavator (Size #4; Nordent Manufacturing Inc., Bonnie Lane, Elk Grove Village, IL, USA). After completing caries removal, the cavity will be washed with sterile normal saline (5 mL) and dried with a sterile cotton pellet. A second sample of dentin will be taken from different sites of the cavity including the walls and floor using a sterile sharp spoon excavator (Size #4; Nordent Manufacturing Inc., Bonnie Lane, Elk Grove Village, IL, USA). The first and second samples will be collected in screw cap sterile vials containing 1 ml of isotonic saline for microbiological evaluation. Following decay elimination by both techniques, cavities will be filled using Glass Ionomer restoration (GC Fuji IX GP®) according to manufacturer's instructions. For Microbiological cultivation and evaluation, dentin samples of all groups will be processed in the Advanced Techniques Laboratory in Dental research within 2 hours of collection. The samples will be vortexed for 30 sec to remove the microorganisms from dentin. The samples will be then regularly watered to obtain a 10^4, and a sterile loop full of sample (0.1 ml) will be collected and cultured with aseptic technique onto blood agar plates (Schaedler agar) by streaking method. Then, the plates will be incubated aerobically at 35^{o}C for 24 hours. Using

colony counter, the total viable count (TVC) will be obtained and expressed as colony forming units “CFU” per ml of sample.

To determine the efficiency of caries removal in all groups, time will be recorded in seconds by using a stopwatch from the beginning of caries removal till the cavity is certain to be caries-free.

To assess the pain experienced throughout caries removal in all groups, the “Wong-Baker FACES® Pain Rating Scale” (Wong & Baker, 1988) will be utilized (Fig. 4).

Dental treatment will be accomplished according to the following steps:

- Local anesthesia will be used for all groups.
- Rubber dam isolation will be used for all groups.
- Caries removal will be done utilizing one of the 3 techniques as follows:

The Control Group: Caries removal using “conventional drilling method”

Caries removal will be carried out using a sterile Tungsten carbide round bur “US-NO 1-8, Meisinger, USA. L.L.C., Easter Avenue Centennial, Colorado” on micromotor handpiece at slow speed (6000-8000 rpm) without water spray. If necessary, undermined enamel will be removed with rotary cutting bur in high speed to enlarge the cavity and provide an adequate access to remove all softened dentin tissue beneath. After caries removal, dentin will be considered caries free utilizing visual and tactile clinical criteria.

The Experimental Group (BRIX-3000): Caries removal using "chemo-mechanical agent" (Brix3000®)

According to manufacturer's instructions, the restorative procedure will be performed without anesthesia under partial isolation with cotton rolls according to the principles of "Atraumatic Restorative Treatment (ART)". Then, the papain-based gel BRIX 3000® will be applied to the cavity with double-ended dental spoon excavator (Size #2, #3, #4; Nordent Manufacturing Inc., Bonnie Lane, Elk Grove Village, IL, USA) leaving it to act for 2 minutes. After 1 minute, the formation of oxygen will be observed and the gel will be turned from translucent green color to cloudy. Then, the infected tissues will be removed through curettage by dental spoon excavator with pendulum movement without pressure, first at the surrounding walls followed by pulp wall removal. After the gel removal, the cavity will be rinsed with water and dried using mild air blow. The cavity will then be evaluated for any residual softened dentin tissue. The procedure could be repeated by applying BRIX3000® as many times as required to remove all of the infected carious tissue. The presence of healthy dentin will be confirmed when (the gel is no longer changed in color and turned turbid. The cavity will be examined according to visual "absence of any discoloration" and tactile "smooth passage of explorer and absence of a catch or tug back sensation" clinical criteria using a dental explorer. Caries will be regarded to be totally removed when the explorer did not stick in dentin and gave no tug back sensation. If necessary, undermined enamel will be removed with rotary cutting bur in high speed "round medium diamond bur size 08-018, Meisinger, USA. L.L.C., Easter Avenue Centennial, Colorado" to enlarge the cavity and provide an adequate access to remove all softened dentin tissue beneath.

The Experimental Group (Carie-Care): Caries removal using "chemo-mechanical agent" (Carie-Care™)

The carious cavity will be treated with Carie-Care™ "Uni-Biotech Pharmaceuticals Private Limited, Chennai, India in collaboration with Vittal Mallya Scientific Research Foundation". Following the instructions of the manufacturer, the Carie-Care™ gel will be brought to room temperature. The gel will be left in the carious lesion for 60 seconds. At that time, the appearance of the gel will be changed from translucent into cloudy. Then the gel will be removed with moistened cotton pellet and softened carious dentin will be scrapped off using spoon excavator ((Size #2, #3, #4; Nordent Manufacturing Inc., Bonnie Lane, Elk Grove Village, IL, USA). The process will be redone till consecutive administration of the gel was unsuccessful to turn into cloudy. Decay removal will be verified by probing with dental explorer (visual-tactile clinical criteria).

Statistical Analysis

The statistical assessment will be carried out using Statistical Package for Social Sciences (SPSS, V.20, IBM; Armonk, NY, USA). Analysis of data will be done through measuring *p*-value to determine statistical significance. The level of significance will be set at 0.05 (*p*-value of less than 0.05 was considered statistically significant). Various statistical methods will be used for measuring *p*-value depending whether the data will be normally distributed (parametric) or not normally distributed (non-parametric). For normally distributed data, paired sample t-test and independent sample t-test will be used. For non-normally distributed data, Wilcoxon signed rank test and Mann Whitney U test will be used.

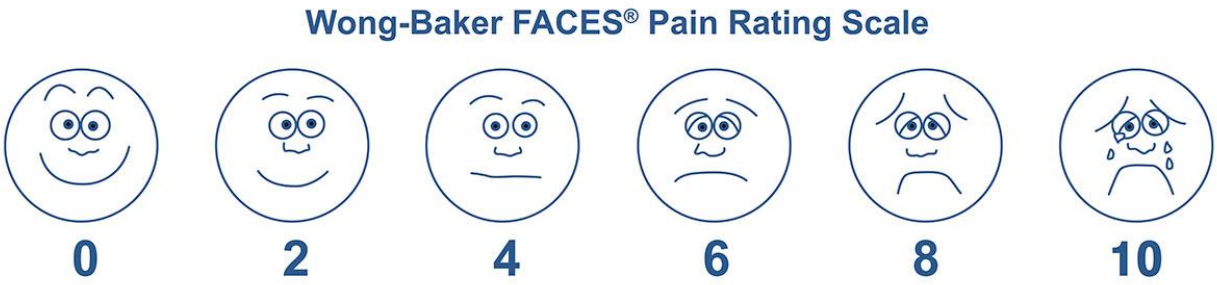

Figure 4: Wong-Baker FACES® Pain Rating Scale**.**

RESEARCH LIMITATIONS

Although, the chemo-mechanical methods cannot eliminate the dental caries completely, it tends to follow the principles of minimal intervention dentistry approaches. The generalization of results is one of the expected limitations of this research.

STRUCTURE OF THE RESEARCH

The research will be divided into several chapters:

- Chapter 1 will represent the introductory note on the state of dental caries among children and treatment approaches that are extensively used by the dentists including different chemo-mechanical agents used.
- Chapter 2 will present the literature review for the study.
- Chapter 3 will depict the methodology for the research. It will provide in-depth explanation of the materials and methods used for gathering data regarding different treatment modalities used in this research.
- Chapter 4 will illustrate the results attained from this research.
- Chapter 5 will provide the discussion of findings on the basis of results obtained in the research.
- Chapter 6 will sum up the entire results with general conclusions, evaluation of hypothesis, policy implications, and recommendations.

THESIS PLAN

STEPS OF PhD THESIS	TIME FRAME (MONTH)											
	1	2	3	4	5	6	7	8	9	10	11	12
Application of CONSORT Statement 2010	X											
Proposal writing		X										
Proposal presentation			X									
Discussion with the statician		X										
Ethical approval				X								
Registration					X							
Apply for funding					X	X						
Providing tools and materials							X					
Intervention for in-vivo study							X	X	X			
Intervention for in-vitro study							X	X	X			
Data analysis									X	X	X	
Thesis writing										X	X	X
Thesis defense												X

EXPECTED FINDINGS

The findings may show an increased efficacy, efficiency with decreased pain experienced during caries removal of chemo-mechanical method using newly developed agent (Brix3000®) as the manufacturer company claimed compared to the previously-available (Carie-Care™) and to the conventional surgical method. It may be recommended as an alternate treatment modality among children, who fear to visit clinics for getting dental treatments.

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